

# Optimisation of biogas production from a microbial perspective

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## Introduction

The hydrolysis of biogas feedstocks and in particular energy crops requires organisms to perform the difficult task of breaking down natural polymers to release accessible carbon mostly as sugars for acid formation. Secondly, methanogenesis, a slower fermentation process is sensitive to acidic pH levels below 7 and oxygen and has different optimal needs to hydrolysis microbial consortia. Current literature searches reveal that studies on wastewater have been performed but not agro-biogas production.

The selected microbial analysis method detects gram positive and gram negative bacteria, actinomyces and fungi using the profile of different phospholipid fatty acids (PLFAs) present in the cell walls of the viable organisms (Baath and Anderson 2003). This analysis is not definitive but provides a multidimensional means of assessing the closeness of different microbial community structures. Other aspects of microbial activity should also be considered, for example, different organisms may function symbiotically in microbial communities mostly because of mutual advantages concerning close proximity including transfer of metabolic products. Biofilms are polymers composed of polysaccharides and smaller amounts of protein and nucleic and humic acids (Jahn and Nielsen 1995). The biofilm protects the organism against changes in temperature, moisture, the chemical environment, microbial predation, to provide structure, adhesion and recognition as well as be able to adsorb nutrients (Virtanen 1951). We did not analysis for the *Achaea* population that can provide acetoclastic and hydrogenotrophic methane production, but they can be profiled by their phospholipid ether linked species present in the cell walls (Gattinger et al. 2003).

Two parallel sets of hydrolysis and methanogenesis tanks were built with one methanogenesis tank containing an immobilising surface in the methanogenesis tank that should support an increasing density of organisms. The support was located in the methanogenesis stage which has been identified as the limiting process in biogas production (Vavilin V.A. et al. 1996). Experimentation involved determining the density and groups of organisms present in both hydrolysis and fermentation stages using molecular diagnostic techniques.

## Materials and methods

Two biogas fermentation systems were identically configured in parallel to compare the effects of different microbial population densities. Process control was achieved through the use of Labview™ software. One methanogenesis tank (tank 4) was fitted with polyurethane foam microbial support media to increase the microbial density. The experiment was operated over several weeks with the accumulation of the mobile phase from each tank. The experiment was initially run to determine the optimum organic loading rate by measuring the alkalinity as being above 4000 mg.l<sup>-1</sup> for both biogas systems.

1 g and 2 g VS.l<sup>-1</sup>.d<sup>-1</sup> (as pig feed) were used for the unsupported and supported systems respectively. The mixers were turned on every hour and the flow rate of the gas mixers was five litres per minute and effectively cycles the entire vessel headspace of five litres during each minute mixing cycle.

The microbial density and microbial community structure was determined using PLFA analysis on samples that were collected twice a day over 8 days. PLFA analysis can determine gram positive and gram negative bacteria, actinomyces and fungi communities by the determination of PLFAs present in the cell walls of only viable organisms. PLFAs are solvent extracted from digestate using the method of (Bligh and Dyer 1959). The extracted PLFAs are derivatised into their fatty acid methyl-esters and analysed on a Hewlett- Packard 5890 II gas chromatograph equipped with a 5973N mass selective detector. The PLFAs i15:0, a15:0, 15:0, i16:0, 17:0, i17:0, cyl7:0, 18:1037, and cyl9:0, were chosen to represent bacterial phospholipid fatty acids (Federle TW 1986); (Frostegard et al. 1993) and 18:2 w6 was used as an indicator of fungal biomass (Federle TW 1986). 10-methyl-C17:0, 10-methyl-C16:0 and 10-methyl-C18:0 represented the actinomyces biomass. The ratio of 18:2w6:bactPLFAs was taken to represent the ratio of fungal: bacterial biomass in soil (Frostegard and Baath 1996).

## Results and Discussion

The PLFA analysis was performed on samples taken twice a day over 8 days. The amounts of PLFAs were cyclical although standard deviations are high in Table 1. The unsupported methanogenesis tank demonstrates the greatest biomass in the mobile phase. This was to be expected as a greater microbial would be supported methanogenesis stage. If the microbial instability can be represented by the relative size of the standard deviation as a fraction of the mean then tank 2 also has the greater instability. The microbial activity in tank 2 was likely to be more unstable than tank 4 because tank 2 would loose microbial biomass and is mostly regulated by hydrodynamic feedstock flows from tank 1.

Figure 1 A boxplot showing PLFAs that contribute most variation from all data

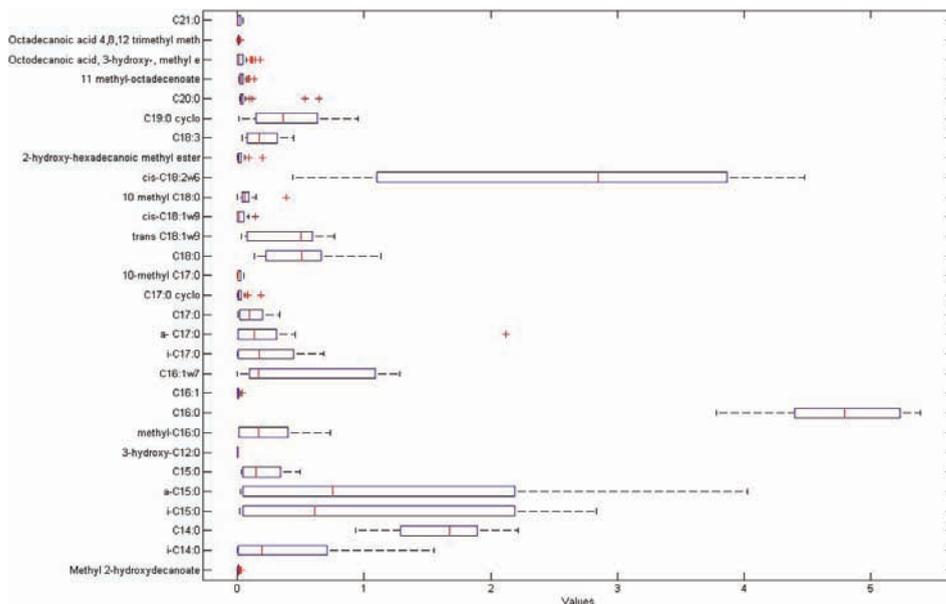
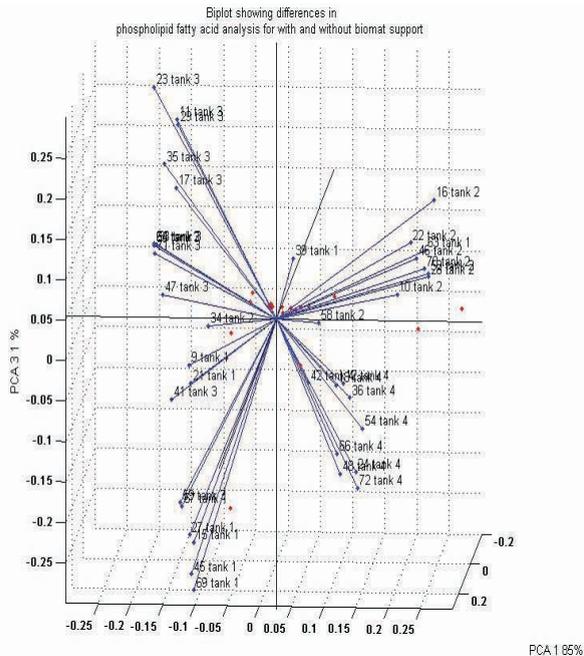


Table 1 Showing the biomass determined by PLFA analysis

	Mean mg.g <sup>-1</sup> dry weight	Standard deviation
tank 1 hydrolysis	244	+91
tank 2 unsupported methanogenesis	543	+509
tank 3 hydrolysis	413	+314
tank 4 supported methanogenesis	389	+250

Figure 1 shows that the major differences are associated with the fungal population C18:2: ω6 and the gram positive bacteria represented by the i and a isomers of C14:0 and C15:0 PLFAs. A biplot of the results (Fig. 2) shows clear differentiation of the 4 tanks from a microbial perspective. Figure 3 shows the microbial content of the hydrolysis tanks 1 and 3 are different and may be attributed to tank 3 having twice the feedstock flow of tank 1.

Figure 2 A biplot showing differences between the microbial community structures for all tanks



In addition to the microbial biomass content there was generally more fungal activity in the hydrolysis tank, where complex organic material was degraded.

There were larger amounts of gram positive organism present in the methanogenesis stage of biogas production.

For the future analysis of the *Archaea* which produce methane and the relationship of biofilm density to biogas production should be investigated. Biofilm measurements are difficult, but near infrared spectroscopy may provide analysis for real process control.

## Conclusion

PLFA analysis shows clear differentiation between the microbial community structure in a biogas system that supports a higher microbial density utilising twice the input material

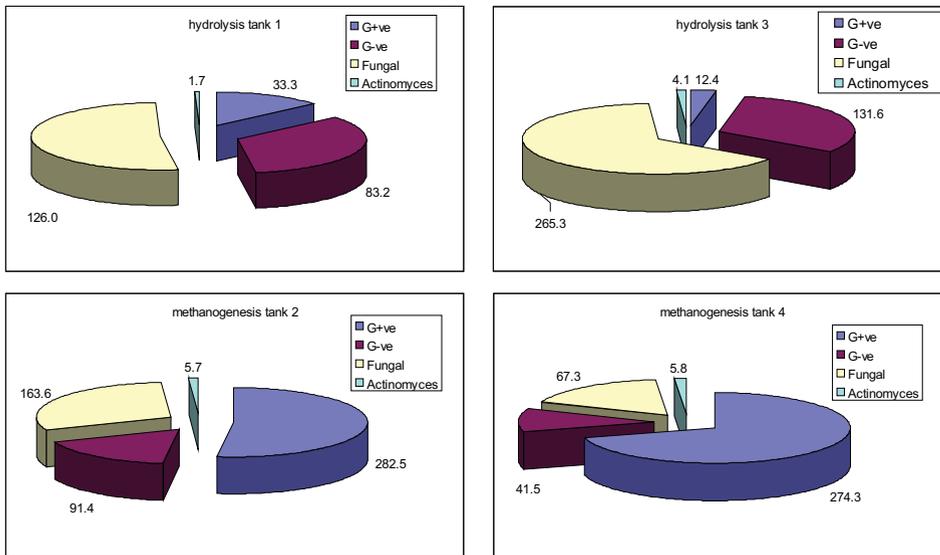
and produces double the biogas output.

Fungal populations are higher in the hydrolysis stage than the methanogenesis stage. There is a higher density of micro-organisms for the system with double the feedstock. There are fewer organisms in the mobile phase for the methanogenesis tank with the microbial support.

The microbial support also aids process stability producing fewer perturbations during microbial cycling.

This study identifies how we can improve biogas yield and reduce capital costs by minimising the volume of the methanogenesis digester.

Figure 3 Microbial community structure of organisms present in the mobile phase of the 2x2 biogas system



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